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Rifat Parveen
Department of chemistry, School of Basic Sciences, SHIATS Allahabad.
Email: parveen.rifat8@gmail.com

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Regioselective synthesis of 1,4-disubstituted 1,2,3-bistriazoles and their antifungal and anti-oxidant evaluation

Prashant Anthony, Nusrat Bashir, Rifat Parveen
Department of chemistry, School of Basic Sciences, SHIATS Allahabad.

Abstract

A series of 1,4-disubstituted 1,2,3-bistriazoles was synthesized via click reacion by cycloaddition of bisalkynes with various organic azides. Synthesized triazoles were characterized by H^1NMR and mass spectral techniques. All the compounds were evaluated for antioxidant and antifungal activities and found to possess moderate to good activities. The antioxidant activity of 1,4-disubstituted 1,2,3-bistriazole derivatives was determined by DPPH(1,1-diphenyl-2-picryl hydrazyl) assay. The antifungal activity was detected by agar well diffusion method against *Aspergillus fumigatus.*

Keywords: Click chemistry, 1,4-disubstituted 1,2,3- bistriazoles, antioxidant activity, antifungal activity.

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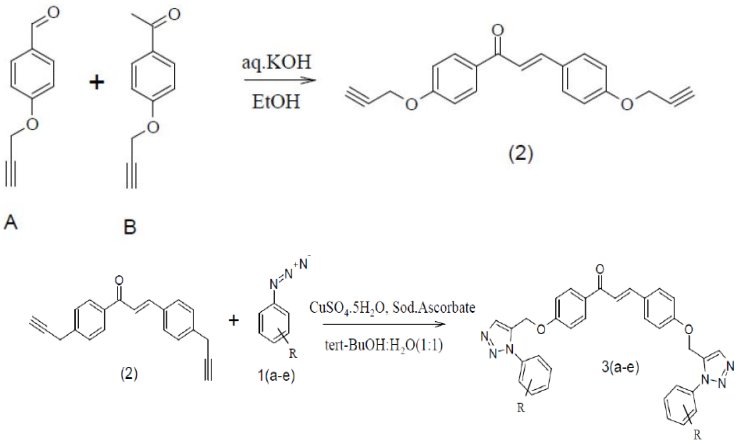
INTRODUCTION:

Heterocyclic ring systems comprise the core of chemical structures of the vast majority of therapeutic agents. Heterocyclic organic compounds containing five or six member ring with one or more nitrogen atoms are of great importance [1]. Triazole and its derivatives represent an important class of heterocycles. They are of biological importance and are used in the synthesis of drugs. The incorporation of various substituents into the 1,2,4-triazole ring and its fusion with various heterocyclic systems yield compounds with enhanced biological activities. The arrangement of 3 basic nitrogen atoms in the triazole ring induces the antiviral activities in the compounds containing this ring [2]. Diazole derivatives are known to exhibit various pharmacological properties such as antimicrobial [3,4], antitubercular [5], anticancer [6,7], anticonvulsant [8], anti-inflammatory, analgesic [9] and antiviral [10]. 1,3-dipolar cycloaddition of azides and terminal alkynes is the most widely used method for the synthesis of 1,2,3-triazoles. Huisgen'S dipolar cycloaddition of organic azides and alkynes is the most direct route to 1,2,3-triazoles. However because of the high activation energy (24- 26 Kcal/mol), these cycloaddition are often very slow even at elevated temperature and produces mixtures of regiosomers. The discovery that Cu (I) efficiently and regiospecifically unites terminal alkynes and azides providing 1,4–disubstituted 1,2,3-triazoles under mild conditions, was a good advance [11,12]. The better regioselectivity, broad scope and the biocompatibility of the compounds have made it one of the most powerful click reaction. The most powerful the Cu–catalyzed azide alkyne cycloaddition (CuAAc) has quickly found many applications in medicinal chemistry [13], supramolecular chemistry [14] and material science [15]. This reaction has been termed the "cream of the crop" of click reactions. Click chemistry has found application in pharmaceutical drug discovery and supramolecular chemistry [16].

EXPERIMENTAL

General methods:

All starting materials were commercially available research grade chemicals and used without further purification. The progress of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25mm (Merck). The chromatograms were visualized under UV 254-366 nm and iodine. Melting points were recorded on Buchi B-545 melting point apparatus and are uncorrected. H^1NMR Spectra were recorded on Bruker Av III HD-300 MHz FT NMR Spectrometer with TMS as internal reference. Mass spectra were performed on JMS-T100LC,AccuTOF mass spectrometer(DART-MS).



*Where R= H, o-Nitro, m-Bromo, m-Methoxy, p-Bromo.

Antioxidant activity

The free radical scavenging activity of the synthesized compounds was studied *in vitro* by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay method[19]. Stock solution of the drug was diluted to different concentrations in the range of 0.4-1mg/mL in DMSO. Test samples dissolved in DMSO (1mL) were added to methanolic solution of DPPH (1mL). The samples were kept in the dark for 30 min after which the optical density was measured at 517 nm with UV Visible spectrophotometer and the percentage of scavenging activity was calculated. Butylated Hydroxy Toluene (BHT) and Gallic acid were used as standard. The inhibition ratio (I %) of the tested compounds was calculated according to the following equation: I % = $(A_0\text{-}A_1)$ / A_0 × 100, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. The concentration of compounds providing 50% scavenging of DPPH•(IC_{50}) was calculated from the plot of percentage inhibition against concentration(µg/mL). All tests and analyses were done in triplicate and the results were averaged.

Anti-fungal activity:

The synthesized compounds were screened for their antifungal activity against *Aspergillus fumigatus* in DMSO by Agar diffusion method [16]. Potato Dextrose Agar(PDA) media was prepared and about 15 mL of PDA was poured into each petriplate and allowed to solidify and 10µL of fungal broth was spread on the surface of PDA plates. A well of 6mm diameter and 50µL volume was bored on the petriplates using a sterile cork borer. The well was filled with test solution(1000µg/mL) prepared in DMSO using a micropippete(20-200µL)

Single well was bored on each plate and two plates were taken for each test compound and the experiment was repeated twice. Fluconazole (10mg/ml) was used as a standard and was introduced into the well instead of the test compound. The plates containing the test organism and the test material in contact were incubated at 27 ± 0.2^{0}C for 48hrs. After incubation the size of the resulting zone of inhibition was measured in mm.

RESULTS AND DISCUSSION

Chemistry:

Structures of compounds **3(a-e)** was confirmed by ^{1}HNMR and mass spectroscopic techniques. The formation of triazoles was apparent due to appearance of characteristic singlet in 1HNMR due to triazolyl protons in the region of δ=7.26.

Pharmacological results:

Anti-oxidant activity

The *in vitro* assay of DPPH scavenging radicals were performed spectrophotometrically with BHT and Gallic acid as standard, and the results are shown in **Table 3.2**. All the compounds showed DPPH scavenger activity with a scavenging effect in the range, 40.29-69.40 % (**3a-e**). Compound **3e** exhibited significant antioxidant activity with IC_{50} value of 56.0 mg/mL when compared to other compounds in the series. All compounds showed more than 50% antioxidant activity at 1mg/mL. The compound 3a showed no antioxidant activity, thus taken as not active.

Compound	m.p (˚C)	Yield (%)	Appearance	TLC	Mol. Formula	Mol. Weight	Recrystillization solvent
3a	130	65	Arylide-yellow solid	Hexane:Ethylacetate(7:3)	$C_{33}H_{26}O_3N_6$	554.61	Diethylether
3b	122	85	Shinydark-golden solid	Hexane:Ethylacetate(7:3)	$C_{33}H_{24}O_7N_8$	644.60	Diethylether
3c	-	75	Brownish oil	Hexane:Ethylacetate(7:3)	$C_{33}H_{24}O_3N_6Br_2$	712.40	Diethylether
3d	-	82	Dark-red oil	Hexane:Ethylacetate	$C_{35}H_{30}O_5N_6$	614.23	Diethylether
3e	140	71	Yellow solid	Hexane:Ethylacetate(7:3)	$C_{33}H_{24}O_3N_6Br_2$	712.40	Diethylether

Table 1: Physical data of synthesized compounds

Tested Compound	Concentration(mg/ml)								IC_{50} mg/ml
	1.0		0.8		0.6		0.4		
	$^{\wedge}_{max}$(517)	I%	$^{\wedge}_{max}$(517)	I%	$^{\wedge}_{max}$(517)	I%	$^{\wedge}_{max}$(517)	I%	
3a	-	**NA**	-	**NA**	-	**NA**	-	**NA**	-
3b	**0.060**±0.001	**55.22**	**0.063**±0.001	**52.98**	**0.074**±0.002	**44.77**	**0.080**±0.001	**40.29**	**0.71**
3c	**0.052**±0.001	**61.19**	**0.064**±0.001	**52.23**	**0.067**±0.001	**50.00**	**0.073**±0.002	**45.52**	**0.60**
3d	**0.041**±0.001	**69.40**	**0.052**±0.001	**61.19**	**0.069**±0.001	**48.50**	**0.077**±0.001	**42.53**	**0.65**
3e	**0.046**±0.001	**65.67**	**0.058**±0.001	**56.71**	**0.063±**0.001	**52.98**	**0.075**±0.002	**44.02**	**0.56**
[a]Gallic acid	**0.383**±0.011	**58.45**	**0.410**±0.006	**55.53**	**0.470**±0.007	**49.02**	**0.532**±0.028	**42.29**	**0.61**
[b]BHT	**0.234**±0.018	**66.42**	**0.265**±0.006	**61.97**	**0.332**±0.011	**52.36**	**0.365**±0.007	**47.63**	**0.45**

Table 2: Antioxidant scavenging activity(%) of synthesized compounds (3a-3e) on DPPH • free radical at different concentrations [a]Gallic acid and [b]BHT(reference antioxidant compounds) were used as a standard. The scavenging capacities were represented as percentage inhibition and values were the means of three replicates **(mean** ± SD,n = 3), NA=Not active.

Antifungal activity:

The *in vitro* antifungal activity of the synthesized compounds (**3a-e**) were studied against *Aspergillus fumigatus*. The results were compared with the standard drug Fluconazole as collected in Table 3.3. Compound **3e** showed significant antifungal activity when compared with other compounds in the series

against *A fumigatus*. Compounds **3b**, **3c** and **3d** were found to be moderately active against tested fungal strain. Compounds **3a** have demonstrated significant antifungal activity comparable to standard. From the results it is evident that most of the compounds showed significant activity and few are moderately active.

S.No.	Compound	*Aspergillus fumigatus* Zone of Inhibition (mm)
1	**3a**	20.00
2	**3b**	19.00
3	**3c**	19.00
4	**3d**	15.00
5	**3e**	21.00
6	Fluconazole	20.00

Table 3: *In vitro* antifungal screening study of the title compounds 3(a-e) well size: 6mm

DISCUSSION:

Compounds **3e** and **3c** showed good anti-oxidant activity in terms of IC_{50} which may be due to the presence of bromo group which increases the penetration of molecules into the lipid membrane so that they increase the antioxidant activity by combining with the reactive oxygen species, which is generated by the different disease conditions [20]**.**

The initial structure activity relationship(SAR) can be drawn for the compounds**(3a-e).** In the present study, different electron withdrawing and electron donating groups attached to phenyl ring as substituent which is linked to triazole moiety were studied for antifungal efficacy. In the present study, it can be revealed that the presence of same substituents at the different positions of the phenyl ring of the series, relatively varies the antifungal and antioxidant activities of **3c** and **3e**. The same substituents in the series and at the different position emphasizes that the electron withdrawing groups increases the potency of the compound and hence **3c** showed relatively more antifungal activity. The compound **3d** showed higher radical inhibition activity which is due to the presence of methoxy group in the aromatic ring [21]**.** The above SAR correlation study reveals that the nature of the substituents on phenyl ring influences the antifungal and antioxidant activities.

CONCLUSION

The research reports the successful synthesis of a series of 1,4-disubstituted 1,2,3-bistriazoles through an easy , convenient Cu(I) catalysed click reaction and evaluation of their *in vitro* antifungal and antioxidant activity. The antifungal and antioxidant activities revealed that all the compounds screened showed moderate to good activities except **3a** in case of antioxidant activity. The significant activities of 1,4-disubstituted 1,2,3-bistriazoles can lead to potential bioactivity. Hence, it can be concluded that, the importance of such work lies in the possibility that the new compounds might be more efficacious drugs against fungi, oxidant activity which could be helpful in designing more potent antifungal and antioxidant agents for therapeutic use.

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